Colony-specific differences in decadal longitudinal body composition of a capital-breeding marine top predator

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ABSTRACT

1. Capital breeding animals such as true seals (*Phocidae*) rely on accumulated body reserves to rear offspring. A mother's body composition at the start of a breeding episode may depend on recent environmental conditions, and sets the resources available for the reproductive episode.
2. At two grey seal (*Halichoerus grypus)* breeding colonies with contrasting demographic characteristics, factors influencing individual variation and temporal trends in the body composition (expressed as the lipid to protein mass ratio) of females were examined.
3. Maternal reproductive expenditure, and the consequences for mothers and their pups, were investigated.

4. Variation in postpartum maternal body composition was considerable. Mean values of 27% (± 5%) lipid and 18% (± 1%) protein were estimated by hydrogen isotope dilution.
5. Mothers with a high lipid to protein mass ratio expended a higher proportion of lipid resources while conserving protein and weaned heavier pups.
6. Average maternal postpartum body composition was similar between the two colonies but declined during the study period at one colony where pup production was decreasing and increased at another where pup production was increasing.

KEYWORDS: behaviour, coastal, mammals, physiology, reproduction

1. INTRODUCTION

Resource limitation forces long-lived iteroparous animals to trade-off survival, growth and reproduction during their lifetime (e.g. Festa-Bianchet, Gaillard, & Jorgenson, 1998; Ronget et al., 2018; Schick et al., 2013; Sheldon & West, 2004). Body composition provides a measure of the immediate phenotypic quality of individuals within a population and can serve as a rough proxy for their relative fitness - animals in 'good' condition can fulfil their own energetic needs and store spare resources for future use, potentially deriving fitness benefits over the long term (Speakman, 2001). Here, 'good condition' is taken to represent some favourable range of body composition which may vary according to sex, age or season; in practice, 'good condition' will be defined by some minimum level or threshold below which growth and / or reproduction become compromised.

Capital-breeding species acquire and store resources over a prolonged period prior to breeding. Marine top predators such as true seals (*Phocidae*) accumulate reserves by exploiting spatio-temporal aggregations of prey (Boyd, 2000; Jönsson, 1997; Stephens, Boyd, McNamara, & Houston, 2009). Physiological mechanisms for energy storage allow for resources to be 'banked' for use during reproductive episodes when feeding does not occur. Within this 'capital-breeding' life history pattern, intrinsic phenotypic and genotypic quality, age and experience may contribute to individual variability in energy resources. Environmental variation (either as prey availability or physical environmental conditions) can

impact body condition and, interacting with intrinsic variability, ultimately affect population demographics (Ferguson et al., 2017; Tartu et al., 2017). It is of fundamental interest to determine the drivers and consequences of intrinsic and extrinsic variability between individuals.

Capital-breeding phocid seals have short lactation periods during which maternal resources are rapidly and efficiently transferred to offspring. Some species enter a total or partial fast during lactation, relying on endogenous energetic capital to fulfil maintenance of metabolic and reproductive costs (Champagne, Crocker, Fowler, & Houser, 2012; Crocker & Costa, 2009). Maternal mass at parturition has emerged as a useful measure predicting lactation performance and strategies, maternal expenditure and offspring size, and future breeding likelihood in some phocid species (Bowen, Iverson, Boness, & Oftedal, 2001; Boyd, 2000; Pomeroy, Fedak, Rothery, & Anderson, 1999; Wheatley, Bradshaw, Davis, Harcourt, & Hindell, 2006). Total body mass is relatively straightforward to measure but important aspects of lactation, pup mass and survival may be better explained with reference to changes in water, lipid and protein body components (Bennett, Speakman, Moss, Pomeroy, & Fedak, 2007; Crocker & Costa, 2009; Mellish, Iverson, & Bowen, 1999; Reilly, Fedak, Thomas, Coward, & Anderson, 1996).

There is considerable debate over which physiological, morphometric or biochemical metrics most accurately and appropriately capture body

composition, and how these may differ between taxa and seasons (Hayes & Shonkwiler, 2001). Typically in field studies, detailed information on body composition is not available and measures of external morphology are used to calculate ratio-based or residual-based body condition indices (Hayes & Shonkwiler, 2001). Gross body mass has also been used to explain reproductive performance in grey seals (Bowen, Iverson, McMillan, & Boness, 2006; Boyd, 2000; Pomeroy et al., 1999). Direct measurement using isotopic methods during the reproductive period allows body components to be quantified (Reilly et al., 1996). Within the breeding episode, the balance of resources available in terms of lipid and protein may govern how those reserves are allocated. Crocker, Webb, Costa, and le Boeuf (1998) found protein use increased with declining adiposity in fasting northern elephant seals and suggested that protein loss could ultimately limit the extent of maternal investment. If energy reserves are depleted too heavily and females leave the colony in relatively poor condition, this may also impact on the success of their subsequent pregnancy (Boyd, 1984, 2000). Pup size at weaning and on departure is positively related to early survival at sea (Bennett et al., 2007; Bowen, den Heyer, McMillan, & Iverson, 2015; Hall, McConnell, & Barker, 2001) and to subsequent recruitment into the breeding population (Bowen et al., 2015) in grey seals. Pups' ability to withstand the post-weaning fast and survive early months at sea may also depend on their mothers' milk composition (Bennett et al., 2007). The composition of stored resources available to the mother may therefore have important consequences for her pup.

105 Logistical difficulties in measuring standardized body composition in free-ranging
106 animals have meant that for many marine mammals, estimates of condition have
107 been obtained from commercial or subsistence hunts (e.g. Hammill & Sauvé,
108 2017; Harwood et al., 2015). While these studies allow direct and complete
109 sampling of the animals, they may suffer from sampling bias and, as cross-
110 sectional samples, cannot impute any longitudinal aspects for individual life
111 histories. In contrast, if non-lethal techniques can be used to obtain condition
112 estimates on animals which can be captured, released, and recaptured, repeated
113 measures can be made in different years (Bowen et al., 2015; Pomeroy et al.,
114 1999). Provided animal life history is not appreciably affected by the
115 measurement process, important additional information about the causes and
116 consequences of changes in individual condition may then be gained from these
117 longitudinal studies.
118
119 Grey seals (*Halichoerus grypus*) are capital-breeding phocids that reproduce on
120 the coastlines of the North Atlantic. Individually identified females have been
121 followed over multiple years at two breeding colonies in the UK, providing
122 information on individual variability in reproductive success (Pomeroy et al.,
123 1999) and body composition. The two study sites are located in regions
124 characterized by different oceanographic features and contrasting population
125 trajectories. North Rona (NR) is located off the UK's Atlantic coast (Figure 1).
126 Pup production has been in decline at this colony since the mid-1990s and there
127 is evidence that recruitment is low (Pomeroy, Smout, Moss, Twiss, & King,

2010). The Isle of May (IM) is located in the central North Sea Here, annual pup production rose steadily between the 1970s and 1990s to stabilize at approximately 2,000 as newer colonies nearby have been formed (SCOS, 2017).

Factors influencing variation in grey seal maternal postpartum body composition, expressed as the ratio of lipid to protein mass, and the consequences for mothers and pups were investigated, comparing individual body composition at NR and IM. Specific objectives were: (1) characterizing inter-annual variation in maternal postpartum body composition; (2) linking changes in individual maternal body composition between breeding episodes; (3) exploring consequences of maternal postpartum body composition for expenditure and for offspring size within a breeding episode.

2. METHODS

2.1 Field sampling

All procedures involving animals in this study were performed under UK Home Office project license 60/4009 or preceding versions and conformed to the UK Animals (Scientific Procedures) Act, 1986. The research was approved by the University of St Andrews Animal Welfare and Ethics Committee. Tritium use was licensed under SEPA regulations.

Lactating grey seals were studied at two breeding colonies: North Rona (NR), Scotland (59°06'N, 05°50'W) and the Isle of May (IM), Scotland (56°11'N,

151 02°33'W) (Figure 1). Individual females were identified by brand, flipper tags or
152 pelage patterns. Parturition and weaning dates for mothers were recorded from
153 detailed behaviour observations from hides. If not directly observed, the birth
154 date of the pup was estimated from indicators of age such as the presence of the
155 umbilicus and its size at first capture. The estimated birth date was the first day
156 of lactation. Weaning was established when the mother had left the pup. Pupping
157 was classed as successful if the pup survived to weaning and weighed more than
158 30 kg by the end of lactation (Pomeroy et al., 1999). All animals included in the
159 present analysis pupped successfully.
160
161 A subset of known females was captured for morphometric measurements and
162 determination of body composition by hydrogen isotope dilution. A total of 122
163 complete captures were available from 2003, 2004, 2007, 2008, 2009, 2010 &
164 2011 at NR and 133 complete captures from 2004, 2007, 2008, 2009, 2011,
165 2012 & 2013 at IM. Each female was captured once in early lactation and again
166 in late lactation. At each capture, females were anaesthetized with a mass
167 specific intramuscular dose of Zoletil 100™ and weighed to the nearest 0.2 kg.
168 Pups were weighed with a spring balance to the nearest 0.2 kg.
169
170 After immobilization of the mother, a 10 mL blood sample was recovered from
171 the extradural vein into heparinized vacutainers to measure background
172 hydrogen isotope activity. A weighed dose (to the nearest 0.1 mg) of tritiated
173 water (HTO) was injected into the extradural vein. The syringe was flushed with

174 blood three times to ensure the entire dose was administered. After an
175 equilibration period of 3 – 5 h, the female was recaptured to obtain a tritium-
176 enriched blood sample (10 mL). All blood samples were centrifuged within 6 h
177 after capture. Plasma was separated and stored at -20°C until analysis.
178
179 **2.2 Hydrogen isotope analysis**
180 Samples collected between 2007 and 2013 were prepared via the distillation
181 method (modified from Ortiz, Costa, & Le Boeuf, 1978; and Arnould, Boyd, &
182 Speakman, 1996). Plasma water was recovered from 200 µl aliquots of blood
183 plasma by direct distillation into pre-weighted plastic scintillation vials; 4 mL of
184 liquid scintillation cocktail (Ecoscint A, National Diagnostics, UK) was added to
185 plasma water after re-weighing vials, shaken for 5 s and then left overnight in a
186 dark refrigerator before analysis. All plasma samples were prepared in duplicate
187 and counted on a Packard 2000 Tri-Carb liquid scintillation counter for 10 min
188 with correction for quenching by external standards. Results were reported as
189 decays per min (DPM), and converted to specific activity ($DPMg^{-1}$) using the
190 mass of recovered plasma water.
191
192 Samples collected between 2003 and 2004 were analysed using whole plasma
193 as per the methods of Pomeroy et al. (1996). Approximately 300 µl of plasma
194 was pipetted into pre-weighed liquid scintillation vials in duplicate. The vial was
195 re-weighed before adding 10 mL of scintillation fluid (Ultima Gold, Perkin Elmer,
196 UK) and left overnight in a dark refrigerator prior to determination of specific

activity via liquid scintillation using the protocol described above. Plasma water content was determined by weighing, drying, and re-weighing a 200 µl aliquot of plasma in duplicate. Specific activity determined in the whole plasma sample was then corrected for water content.

To test if the two methods of preparing samples introduced significant differences in the estimation of specific activity, the following protocol was used. A sample of plasma was obtained from a captive grey seal at the Sea Mammal Research Unit. The sample was artificially enriched with a quantity of HTO injectate and diluted to mimic the approximate *in vivo* dilution (1:20,000). The sample was split and 25 subsamples were prepared for liquid scintillation analysis using each of the two methods described above (n = 25 samples using each method). An analysis of variance test was used to test for significant effect of preparation method. There was no significant effect of preparation method on the specific activity of an artificially enriched plasma sample ($F_{(1,48)}$ = 0.263; *MSE* = 48895; *p* = *0.61*), suggesting that it was unlikely that differences in 2003 and 2004 results compared to 2007:2013 results were due to the different methods of analysis.

Standards for the injectate from the year of the study were prepared by gravimetric dilution of unused injectate to mimic approximate *in vivo* dilution. 200 µl of the diluted injectate was added to 4 mL of liquid scintillation fluid (EcoScint A, National Diagnostics, UK) and counted in the same manner as plasma samples.

220

221 **2.3 Maternal body composition calculations**

222 Body composition was estimated according to the methods of Reilly and Fedak
223 (1990). Dilution space, *D*, was calculated from the mean specific activity of the
224 seal plasma duplicates and injectate samples according to equation (1):

225

226 (1) $$D_{HTO} = \left(\frac{SA_{inj}HTO_{admin}}{SA_{eq} - SA_{ini}}\right) 0.001$$

227

228 where SA_{inj} is the specific activity of the injectate for the year of sampling,
229 HTO_{admin} is the dose of tritiated water administered to the seal and SA_{eq} and SA_{ini}
230 are the specific activity of equilibrium and initial (background) samples,
231 respectively. Reilly and Fedak (1990) determined an empirical relationship
232 between isotope dilution space and total body water (TBW) from four grey seals
233 (two mother-pup pairs) which was used to correct D_{HTO} to TBW.

234

235 (2) $$TBW = -0.234 + 0.971 D_{HTO}$$

236

237 Evidence suggests that the rate of total body mass loss is relatively constant over
238 the lactation period (Fedak & Anderson, 1982). The daily rates of water and
239 mass loss (kg d^{-1}) were assumed to be constant and were calculated from the
240 change between early and late lactation captures. Median number of days
241 between captures was 11 days (range: 6 to 17). Maternal postpartum mass
242 (MPPM) and TBW were extrapolated by adding the product of the daily mass

loss and the number of days since parturition to mass at first capture. Similarly, the product of the daily mass loss and the number of days between last capture and weaning was subtracted from mass at last capture to extrapolate to maternal wean mass (MWM) and TBW at weaning. Proportion body water, P_{water}, postpartum and at weaning was calculated using extrapolated masses.

Equations from Reilly and Fedak (1990) were used to predict proportion lipid (Eqn 3) and protein (Eqn 4) using observed P_{water} values within the range of the original Reilly and Fedak (1990) data (P_{water} =[0.43, 0.68]).

(3) $$P_{lipid}\ (\%) = (105.1 - 1.47 \times P_{water}(\%)) \times MASS\ \text{(kg)}$$

(4) $$P_{protein}\ (\%) = (0.42 \times P_{water}(\%) - 4.75) \times MASS\ \text{(kg)}$$

where P_{water} and *MASS* refer to these measurements at parturition and at weaning. Masses of lipid and protein components at the beginning and end of lactation were calculated using estimated MPPM and MWM. To compare lipid and protein together across individuals a metric of body composition that reflected animal fatness relative to lean mass was calculated. Maternal composition was expressed as the ratio of lipid mass to protein mass at the beginning (maternal postpartum condition, MPPC) and at the end of lactation (maternal weaning condition, MWC). Relative expenditure of lipid and protein over the breeding episode was estimated as the difference between masses of

respective body components at postpartum and at weaning divided by their postpartum mass. Acronyms of all body composition metrics and their meaning are given in Table 1.

2.4 Data analyses

MPPC was determined for all mothers in the study. However, some covariates were not always available for every study animal (e.g. weaning day, age). Furthermore, while body composition observations for some mothers occurred in several years most did not occur in consecutive years and observations from other mothers occurred only once. Because information about all covariates for every observation was not available to include in a single model, data subsets were used which maximized the information available for each dependent / covariate set of interest (see Table 2 for details of sample size used in each analysis).

Temporal trends in all observations of MPPC at IM and NR were characterised by fitting a generalized additive mixed model (model M1) to annual postpartum body composition data using a Gaussian error distribution. Year was included as a smooth term for each colony and individual was specified as a random effect. Visual inspection was used to detect any significant temporal autocorrelation in model residuals.

The effects of mother age and of immediate past reproductive history on MPPC were tested (model M2). This was possible only for a subset of animals, where data were available. Age was not included in M1 because there was an imbalance in the number of animals that had been aged in each year between NR and IM. At NR, the number of sampled known age mothers declined from n = 17 in 2003 to n = 2 in 2011. At IM, the number of known age mothers was between 10 and 20 in each year. Therefore the effect of maternal age on MPPC was considered separately in a linear mixed effects model, M2, along with information on reproductive state in the previous breeding episode which was available for all known age mothers. A quadratic term was included to allow for a non-linear relationship between age and MPPC. The impact of reproductive state in the previous year on MPPC was examined by including a breeding state covariate ('pup') coded as 1 (individual was seen on the colony and weaned a pup successfully in the previous year), or 0 (all other conditions). An interaction between age and the categorical covariate of colony (IM, NR) allowed for different age-related effects at each site. To account for potential annual effects specific to each colony a random intercept term of year within colony was added. An additional random intercept for each individual was included to estimate the general nature of variation among individuals while allowing for repeated measures on some individuals.

For a limited number of mothers, body composition samples were available in consecutive breeding episodes providing an opportunity to assess longitudinal

311 changes in expenditure and body composition. The relationship between relative
312 expenditure in terms of lipid (LEXP) or protein (PEXP) and the difference in
313 postpartum lipid (ΔMPPL) or protein mass (ΔMPPP) between consecutive
314 breeding episodes (models M3 and M4) was examined to investigate if high
315 expenditure of lipid or protein in one year incurred a cost to the mother in the
316 following breeding episode in terms of reduced absolute postpartum lipid or
317 protein mass. The model was fitted with a generalised additive model after initial
318 data inspection suggested non-linear relationships. A fixed effect for colony (IM,
319 NR) was included to allow for any overall difference in expenditure between
320 colonies.
321
322 Having explored potential drivers of variation in postpartum body composition,
323 the consequences of that variation within a breeding episode was explored next.
324 Mothers in better condition may be more capable of expending a larger
325 proportion of lipid stores during a breeding episode and also be capable of
326 sparing protein by meeting the majority of energetic needs with lipid (Crocker et
327 al., 1998; Fedak & Anderson, 1982). The relationship between MPPC and the
328 relative expenditure of each body component (LEXP, PEXP) within a breeding
329 episode was characterized using a mixed effects model with a random effect for
330 individual and year nested within colony (models M5 and M6). A difference in
331 expenditure between colonies was tested for by including a fixed effect for colony
332 (IM, NR). MPPM was included in both models to account for any effect of
333 maternal size on MPPC.

Relative expenditure of lipid and protein contributes to variation in maternal body composition at weaning (MWC). Mothers expending a higher proportion of their resources, and those leaving the colony in poorer condition, might incur a cost to reproductive output (attendance and pupping) the following year (Boyd, 2000; Pomeroy et al., 1999). The association between MWC in one year and the probability of producing a pup in the subsequent year was explored using a mixed effects logistic model. Random effects were included to take account of variation among individuals and years (model M7).

To explore how maternal state at parturition influenced one measure of pup quality – pup weaning mass (PWM) – body composition, relative lipid expenditure, postpartum mass and maternal age were included in a multiple regression with PWM as the response variable (model M8). Average PWM was allowed to vary in IM and NR by including a fixed effect for colony. Covariates were included in a linear mixed effect model with a random effect for individual and year nested within colony. A Gaussian error distribution was specified. Multi-collinearity in the predictor variables was assessed by calculation of variance inflation factors (Fox & Weisberg, 2011).

Annual colony average MPPC estimates from model M1 were related to independent survey estimates of pup production at each colony (SCOS, 2016)

356 using a general linear model with Gaussian error distribution (M9) to determine if
357 average maternal condition was related to trends in local pup production.
358
359 The *lme4* package was used to fit linear mixed effects models (Bates, Mächler,
360 Bolker, & Walker, 2015) and the *mgcv* package (Wood, 2011) to fit generalised
361 additive mixed models in the R statistical package (R Core Team, 2018).
362 Determining the degrees of freedom and p-values in mixed effects models is not
363 straightforward (Bates et al., 2015). For those fitted with lme4, the significance of
364 terms within the model was evaluated by profiling of 95% confidence intervals
365 (CI) on parameter estimates. Where 95% CI encompassed zero, the term was
366 not considered significant at the 5% in explaining variation in observations.
367 Marginal ($R^2_{GLMM(m)}$; fixed effects only) and conditional ($R^2_{GLMM(c)}$; fixed and
368 random effects) deviance explained was assessed for each fitted model
369 (Nakagawa & Schielzeth, 2013) implemented in the *MuMIn* package in R
370 (Bartoń, 2016). All continuous variables were centred to aide in model
371 convergence and all statistical analyses were performed in the R statistical
372 programme (R Core Team, 2018).
373

374 **3. RESULTS**

375 Summary statistics of body measurements can be found in Table 2. Postpartum
376 mass was higher at NR than IM but average body compositions were not
377 significantly different (Table 2). A typical postpartum grey seal mother in our
378 dataset was ca. 27% lipid, 18% protein and 53% water with the remaining 2%

attributable to mineral components. Therefore an 'average seal' immediately after parturition had ca. 1.5 times more lipid than protein (i.e. MPPC = 1.5). At weaning, an average mother's body composition was ca. 15% lipid and 21% protein, giving an MPPC ca. 0.75.

Non-linear trends in maternal MPPC were different between the colonies and included lower values at IM in 2004 and higher values at NR in 2003 & 2004 (Figure 2; M1, Table 3). There was a contrast in the temporal trends in MPPC at the two colonies: the year(s) with high MPPC at IM (2009, 2011) corresponded to the lowest MPPC years at NR while the lowest MPPC year at IM (2004) corresponded to one of the highest at NR.

A subset of mothers for which MPCC was estimated were of known age, and had information on presence and pupping in the previous year (n = 111 observations from 59 mothers at IM and n = 53 observations from 26 mothers at NR). When reproductive state in the previous year and a non-linear effect of age were included in a mixed effects model of MPPC, there was no significant effect of either covariate and no evidence for a difference between IM and NR (M2, Table 3).

A total of 38 consecutive observations of individual MPPL and MPPP were available from 29 mothers. There was a significant overall negative relationship between PEXP and ΔMPPP. Mothers that expended a high proportion of their

postpartum protein mass (>25%) during a breeding episode returned the following year with smaller postpartum protein stores, although some exceptions were evident (Fig. 3). This relationship did not differ significantly between IM and NR (Figure 3; M4, Table 3) although PEXP was, on average, greater at NR (Table 2). In contrast, the change in lipid mass between consecutive breeding episodes was not significantly related to lipid expenditure at either IM or NR, suggesting little evidence for a tangible cost to subsequent body composition from high expenditure of lipid, nor of a benefit from lipid sparing.

Mothers that had larger amounts of lipid relative to protein mass at postpartum expended relatively more of their lipid within the breeding episode. A one-unit increase in MPPC was associated with an increase in LEXP expenditure of 18.29% (95% CI [7.31, 27.80]) (Figure 4a; M5, Table 3). Protein use over the lactation period was negatively associated with MPPC (Figure 4b; M6, Table 3). A one-unit increase in MPPC resulted in a reduction in PEXP of 10.6% (95% CI [-14.42, -6.46]). Thus, one consequence of low MPPC was a greater use of protein over the breeding fast.

Despite the consequences of relative maternal body composition at postpartum for overall expenditure (on pup and to meet maternal metabolic demands) within the breeding episode, there was little evidence that mothers leaving the breeding colony in poor condition (low MWC) were less likely to attend the colony in the following breeding episode at either IM or NR (p-values all > 0.7; M7, Table 3).

Pup weaning mass was positively associated with maternal postpartum mass in the two colonies but similarly-sized mothers produced heavier pups at NR than at IM (Figure 5; M8, Table 3). On average, for every 10 kg increase in MPPM, PWM increased by an average of 1.6 kg and pups at NR were 7.9 kg heavier. Maternal postpartum condition, relative lipid expenditure and maternal age were not significant predictors of PWM. Variance inflation factors were less than 2 for each parameter included in the model.

Although based on a limited number of years, the annual fits of MPPC from M1 were significantly and positively related to pup production at NR but not IM (M9, Table 3).

4. DISCUSSION

Empirical estimates of body composition of a capital-breeding pinniped were obtained from an individual-based long-term study at two UK breeding colonies. Temporal trends in maternal condition differed between two grey seal colonies with contrasting trends in pup production, but the effect of intrinsic factors on individual body composition or expenditure appeared to be similar. There was no evidence of an age effect on maternal body composition. Despite a similar relationship between maternal postpartum body composition and body

447 component expenditure at the colonies, average pup weaning masses at IM were
448 less than those achieved by similarly-sized mothers at NR.
449
450 **4.1 Individual variation in maternal body composition**
451 In this study grey seal MPPM ranged from 121 kg on IM to 258 kg on NR and
452 lipid mass at parturition ranged from 47 kg at IM to 70 kg at NR. Clearly the
453 absolute resources available to breeding females at these extremes are very
454 different and set proximate limits on potential pup weaning masses. Determining
455 the variation in mothers' stores of lipid and protein components in animals of
456 markedly different sizes was made accessible using the relative measures
457 MPPC, LEXP, PEXP.
458
459 No significant relationship was detected between age and measures of body
460 composition from the subset of known age females in the present analysis (M2),
461 even though a quadratic age term allowed for potential differences in body
462 condition between early years, prime breeding years and senescence. Evidence
463 of age-related changes in maternal body mass and composition from other
464 capital-breeding pinnipeds is equivocal. There was little evidence for an
465 association between maternal age and postpartum lipid content of 40 female
466 Weddell seals (Wheatley et al., 2006), and Pomeroy et al. (1999) found no
467 association between inter-annual changes in MPPM and age of female grey
468 seals at NR. In a long-term study tracking individual female grey seal
469 reproductive performance over several years, Bowen, Iverson, McMillan, and

Boness (2006) demonstrated declines in multiple measures of reproductive performance in older mothers but noted the findings could not be explained as the result of reduced maternal body condition. In view of the lack of significant age-related effects on MPPC for the subset of aged mothers in the present analysis, the inter-annual changes in MPPC seen in the larger dataset (M1) are unlikely to be due simply to differences in the ages of mothers included in the study.

The present showed a positive association between maternal postpartum mass and pup wean mass, as did Pomeroy et al., (1999). However, neither MPPC, LEXP, maternal age, nor pup sex were significant in a full model to explain PWM, which also included MPPM (M8). This is perhaps unsurprising given that mass is only one of several measures of pup quality. Mothers at NR produced heavier pups at higher MPPM than did those at IM (Figure 5). NR mothers were on average heavier than those at IM and it is likely that they gave birth to heavier pups (Fedak & Anderson, 1982), accounting for some of the offset in pup weaning mass. While pup size has been positively related to early survival at IM (Hall et al., 2001), it would be interesting to know if this result were generalizable across pups from different colonies or if a similar but separate relationship exists for those, larger, animals on the Atlantic coast. Body composition of pups was not available for this study but is likely to also be important in early development and for survival (Bennett et al., 2007). In the present study, maternal postpartum body composition did not appear to be a better indicator of pup quality (as

493 measured by mass) than maternal postpartum mass. Thus, at least within a
494 colony, maternal postpartum mass may be a sufficient proxy for predicting
495 potential pup size.
496
497 **4.2 Longitudinal effects – change and consequences**
498 Sequential observations of free-ranging seals body composition are rare in the
499 literature. In this study, pupping success in the previous year did not significantly
500 affect MPPC (M2). However, the present study assumed that females not seen
501 on the colony also did not pup in the non-observed breeding episode. While
502 breeding site fidelity has been high at these colonies (Pomeroy, Twiss, &
503 Redman, 2000), it is possible that females not observed on the colony pupped
504 successfully elsewhere.
505
506 Previously, grey seal mothers at NR showing large proportional mass
507 expenditures during lactation in one year were associated with a decrease in
508 MPPM the following year (Pomeroy et al., 1999). Those results were extended
509 here to show the different allocations of body components during lactation. In the
510 subset of mothers measured in successive years, those that lost a larger
511 proportion of their postpartum protein mass in one breeding episode had
512 relatively smaller postpartum protein stores in the following season (M4).
513 Changes in postpartum protein mass were less than those of lipid mass, in
514 keeping with the need to conserve protein to meet basal metabolic demands
515 (Champagne et al., 2012; Costa, 2009). Mothers may modulate resource

allocation to breeding based on their body composition state at the start of the breeding season, which is affected by foraging success over the inter-breeding period as well as the allocation of resources to reproductive expenditure in the previous year.

Importantly, there were no significant differences in the relationships detailed above between IM and NR suggesting that individual mothers at a growing (IM) and declining (NR) colony used body resources similarly to produce pups. This may signal that differences in the marine environment where these animals forage, rather than differences in the intrinsic factors affecting maternal body composition (such as age or past reproductive allocation of resources), could be responsible for the colony-specific temporal patterns in maternal body composition.

Results from the longitudinal aspect of the present study linked variation in maternal expenditure in one breeding episode to maternal resources in the following year. Within a breeding episode, MPPC was an important determinant of maternal expenditure in terms of both lipid and protein mass (M5 & M6). The present study found the amount of protein utilized over the breeding season was negatively related to initial MPPC: mothers with more lipid relative to protein at the start of the breeding episode season utilized less of their protein reserves (relative to body mass) during lactation. This evidence of increased protein conservation in animals with higher initial fat reserves is consistent with evidence

539 from several other pinniped species (see references in Champagne et al., 2012).
540 Such mothers were able to lose proportionately more of their mass in terms of
541 lipid. Maternal mass and resource transfer efficiency is high in this species
542 (Fedak & Anderson, 1982; Pomeroy et al., 1999; Reilly et al., 1996). This would
543 suggest that mothers with a high MPPC at the start of the lactation period are
544 able to expend more on their offspring, potentially weaning a fatter pup.
545
546 Females must balance the drive to maximize expenditure on their offspring with
547 the conflicting demands of their own metabolic maintenance both during
548 lactation, and after leaving the colony. Females leaving the colony with low lipid
549 reserves relative to protein mass may be less likely to return to the colony the
550 following season because poor condition has been related to delayed
551 implantation (Boyd, 1984). However, there was little evidence that MWC affected
552 the odds of an individual returning to either colony the following year from the
553 present data (M7). The same considerations about equating absence at the
554 colony with a failure to pup discussed above also pertain here. Non-returning
555 females may have gone elsewhere to breed, or skipped a breeding episode; the
556 physiological or behavioural factors involved in these two outcomes may be quite
557 different. Without the ability to track departing females through to the next
558 breeding season, it is not easy to make the potentially important distinction
559 between them.
560
561 **4.3 Colony level effects of body composition**

562 Mean maternal postpartum body composition at NR and the IM showed different
563 temporal trends although there was considerable intra- and inter-year variation at
564 both colonies (M1, Figure 2). The lack of temporal correspondence in trends of
565 average body conditions (Figure 2) suggests that there is no single common
566 environmental driver on maternal condition and that colony-level trends could be
567 related to local scale environmental variation altering prey availability and seal
568 foraging success in the inter-breeding interval. Adult female grey seals around
569 Britain are most likely to forage in the region containing their breeding colony
570 (Russell et al., 2013). The marine characteristics surrounding NR in the NE
571 Atlantic, and the IM, within the North Sea, are substantially different. As long-
572 lived and wide-ranging predators, seals should be buffered from short-term or
573 local fluctuations in foraging success. Nevertheless, long-term and broad-scale
574 consequences of environmental change have been documented in several
575 pinniped species. The long-term decline in number and body size of Steller sea
576 lions (*Eumetopias jubatus*) in the Gulf of Alaska is thought to be at least partially
577 due to nutritional stress due to reduced availability of high-quality prey and their
578 replacement by less nutritious species (Trites & Donnelly, 2003). Variation in
579 harp seal blubber thickness in the Barents Sea has been related to the
580 abundance of different prey groups (Øigård, Lindstrøm, Haug, Nilssen, & Smout,
581 2013). Ferguson et al. (2017) found long-term declines in ringed seal blubber
582 thickness related to changing marine conditions in the Hudson Bay (longer
583 periods of open water, ENSO index and NAO index). The authors of the latter
584 study noted concomitant changes in ringed seal diet composition and a general

585 decline in pup production in the region. Long-term monitoring of southern
586 elephant seals on Macquarie Island has demonstrated links between
587 environmental conditions and maternal condition and expenditure; smaller
588 mothers tended to invest relatively more in male pups during favourable years,
589 providing increased likelihood of their pups surviving the first year (McMahon,
590 Harcourt, Burton, Daniel, & Hindell, 2017). The present study found a decline in
591 maternal postpartum condition between 2003/2004 and subsequent years at NR
592 was associated with declining pup production, and which may also be linked to
593 changes in environmental conditions impacting foraging success, similar to that
594 described for southern elephant seals (e.g. Hindell et al., 2017).
595
596 Over the period of this study, grey seal pup production growth has slowed and
597 stabilized in the Hebrides while continuing to grow in the North Sea region
598 surrounding IM, particularly to the south (SCOS, 2017). Colony level
599 demographics at IM and NR reflect these changes: pup production at IM
600 increased by 17% from 1,953 in 2004 to 2,355 in 2012, and declined by 44%
601 from 970 in 2003 to 547 in 2012 at NR. Estimates of apparent survival and
602 fecundity are lower for females breeding at NR than at IM, and NR has lower
603 recruitment, consistent with the trend in pup production (Pomeroy et al., 2010,
604 Smout et al., 2011). Within observations from NR, the present study found a
605 positive relationship between average MPPC of monitored females and colony-
606 level pup production. Although average MPPC at NR declined, the mothers
607 included in the sample raised pups successfully in the years they were observed

608 at the colony. This decline in average condition of successfully breeding animals
609 may point more towards a decline in the number of animals managing to breed at
610 this colony, consistent with the hypothesis that environmental conditions for
611 these animals during foraging no longer produce sufficient resources to support
612 the historical breeding population, as suggested by lower fecundity estimates at
613 NR (Smout et al., 2011). The lack of association between average MPPC and
614 pup production at IM might be explained if in general, females breeding there
615 have access to and are successful in obtaining adequate food resources such
616 that their body composition, while showing some temporal variation overall, was
617 'sufficiently good' over the period of the study. Continued recruitment of new
618 breeders, consistent survival rates and adequate fecundity of breeding females
619 could then support continued growth of the IM colony and its neighbours.
620
621 Where seal populations are monitored to determine population status and trends,
622 destructive sampling (often from commercial hunts) offers cross-sectional
623 information, not just for demographic parameters such as age-specific survival
624 and fecundity but also for condition measures, including morphometrics such as
625 mass, length, girth and typically, blubber thickness or sculp mass. While blubber
626 thickness may be an index of seal condition, this gross measure ignores non-
627 blubber fat and must be taken from seals of the same age/reproductive state to
628 be informative. Non-destructive sampling such as that described here is more
629 labour intensive, and long term longitudinal studies require sustained support.
630 They have enabled a more complete assessment of the factors involved in

631 determining the variation and outcomes of MPPC at individual and colony levels,
632 revealing the effects of individual aging and sequential reproductive effort
633 (Bowen et al., 2006; Pomeroy et al., 1999). Some inter-individual variation in
634 MPPC may be due to past condition, but annual patterns in condition metrics at
635 the colony level may be indicators of environmental changes affecting foraging
636 success and the carrying capacity of the environment for the local seal
637 population.
638
639 NR mothers weaned large pups whose survival would be expected to be high,
640 but there has been little or no recruitment at NR (Pomeroy et al., 2010).
641 Therefore post-weaning factors (survival or emigration) must influence
642 recruitment to the natal breeding colony as much or more than PWM alone.
643
644 This study highlights the benefit of long-term, longitudinal monitoring at multiple
645 sites to contextualise patterns in conservation data. Detailed investigation of
646 individual quality, resource allocation and offspring production allows for the
647 mechanisms of observed changes in population dynamics to be better
648 understood. This demonstrates the difficulty of producing simple indicators of
649 population status or individual quality: without context, in the broadest ecological
650 sense, they will be of limited value.
651

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817 **Table 1:** Definition of grey seal (*Halichoerus grypus*) body composition acronyms
818 used in the text and data analysis.
819

Abbreviation	Meaning
MPPM	Maternal postpartum mass (kg)
MWM	Maternal weaning mass (kg)
MPPL	Maternal postpartum lipid mass (kg)
MPPP	Maternal postpartum protein mass (kg)
MPPC	Maternal postpartum condition (MPPL/MPPP)
MWC	Maternal weaning condition (mass lipid at weaning/mass protein at weaning)
LEXP	Lipid expenditure (% MPPL)
PEXP	Protein expenditure (% MPPP)
PWM	Pup weaning mass (kg)

820
821
822

823 **Table 2:** Summary statistics of female grey seal morphometric and body

824 composition data.

825

Metric	Isle of May (mean [95% CI])	n	North Rona (mean [95% CI])	n
Postpartum mass (MPPM, kg)	179 [175, 183]	134	190 [187, 194]	122
Age[1]	18 [8, 32]	116	18 [10, 32]	58
Postpartum lipid (%)	26.5 [25.6, 27.4]	134	26.9 [26.0, 27.8]	122
Postpartum protein (%)	17.7 [17.5, 18.0]	134	17.6 [17.3, 17.9]	122
Weaning lipid (%)	14.9 [13.5, 16.2]	94	15.3 [13.7, 17.0]	93
Weaning protein (%)	21.0 [20.6, 21.4]	94	20.9 [20.4, 21.3]	93
Postpartum condition (MPPC)	1.53 [1.46, 1.61]	134	1.56 [1.49, 1.64]	122
Weaning condition (MWC)	0.74 [0.66, 0.82]	94	0.79 [0.68, 0.89]	93
Lipid expenditure (LEXP, % MPPL)	60.8 [56.8, 64.9]	94	61.6 [57.3, 66.0]	93
Protein expenditure (PEXP, % MPPP)	20.3 [18.7, 21.9]	94	23.3 [21.2, 25.3]	93
Pup wean mass (kg)	43.7 [42.4, 45.1]	86	52.5 [50.9, 54.0]	92

826 [1] Age given is median [minimum, maximum]

827

828

Table 3: Details of fitted models, method used, and results. Regression parameters are mean [95% confidence intervals]. Year of observation is indicated by the subscript *i*. Variance explained by fitted model is given by R^2 value. For linear mixed effects models, marginal R^2_m indicates variance explained by fixed effects alone and conditional R^2_c is an estimate of variance explained including random effects (Nakagawa & Schielzeth, 2013). In the model specifications, 's()' indicates a smooth function; 'x' indicates an interaction term and '/' indicates nesting.

	Response	Fixed covariates	Random effects	n	Significant term(s)	p-value	β	R^2_m	R^2_c
M1	MPPC	s(Year) : Colony	Individual (n = 138)	255	s(year):IM s(year):NR	0.019 < 0.001		40	
M2	MPPC	(Age + Age^2) x Colony + $Pup_{[I+1]}$	Individual (n = 85), Year/Colony (n = 16)	164				6.6	42
M3	ΔMPPL(%)	s($LEXP_{[i-1]}$) + Colony		38				22	
M4	ΔMPPP (%)	s($PEXP_{[i-1]}$) + Colony		38	s($PEXP_{[i-1]}$)	0.012		15	
M5	LEXP	MPPC x Colony + MPPM x Colony	Individual (n = 111), Year/Colony (n = 15)	187	MPPC		18.29 [7.32, 27.80]	11	30
M6	PEXP	MPPC x Colony	Individual (n = 111), Year/Colony (n = 15)	187	MPPC		-10.62 [-14.42, -6.46]	28	49

M7	$Pup_{[I+1]}$	MWC x Colony	Individual (n = 107), Year/Colony (n = 15)	177			< 1	< 1
M8	PWM	MPPC x Colony + MPPM x Colony + LEXP x colony + Maternal age	Individual (n = 69), Year/Colony (n = 15)	117	Colony MPPM	7.86 [3.80, 12.12] 0.16 [0.11, 0.22]	47	74
M9	MPPC (M1) at IM	Pup production at IM		8				
	MPPC (M1) at NR	Pup production at NR		8	Pup production at NR	384 [118, 797]	19	

Figure 1: Map of the United Kingdom showing the location of the two grey seal breeding colony study sites, Isle of May (IM) in the North Sea and North Rona (NR) in the Atlantic.

Figure 2: Generalised additive mixed model (GAMM) smoothed temporal patterns in mean postpartum condition (MPPC, defined as the ratio of lipid to protein mass) of breeding female grey seals at Isle of May (IM) and North Rona (NR) colonies (line and 95% confidence intervals in grey). Circles represent observed values; filled circles indicate samples from known age mothers.

Figure 3: The relationship between change in postpartum protein mass in consecutive breeding episodes ΔMPPP and the proportion of protein expended PEXP in the first of those years.

Figure 4: Relationship between the proportion of lipid mass loss (LEXP) and maternal postpartum condition (MPPC) (a); and the proportion of protein mass loss (PEXP) and maternal postpartum condition (MPPC) (b).

Figure 5: Grey seal pup weaning mass (PWM, kg) increased with maternal postpartum mass (MPPM) at both the Isle of May (IM, solid line and filled triangles) and North Rona (NR, dashed line and open circles) breeding sites.